consisting of said serine residue at said phosphorylation site and a plurality of amino acid residues before and/or after said phosphorylation site, and said antibodies specifically recognizing said phosphorylation site of said partial peptide.

24. A method for detecting Alzheimer's disease comprising reacting one or more antibodies from the reagent kit according to claim 23, with a body fluid sample from an individual suspected of having Alzheimer's disease, to detect from the reactivity of said antibodies whether said individual has Alzheimer.

REMARKS

Favorable reconsideration is respectfully requested in view of the foregoing amendments and the following remarks.

Claims 1, 6-11 and 15-17 have been amended to put the claims in better form under U.S. practice and to more particularly define the present invention. Further, new claims 18-24 have been added to further protect the present invention. Applicants wish to note that unless otherwise specifically recited below, the claim amendments are merely editorial in nature and should not be construed to limit the scope of the claims. Support for the claim amendments and new claims is readily apparent from the teachings of the specification and the original claims.

With regard to the rejection of claims 1 and 6-17 under 35 USC § 112, second paragraph, as set forth in item 10 of the May 9, 2001 Official Action, this rejection has been overcome by the amendments to the claims which address each ground of rejection set forth by the Examiner.

Specifically, the amended claims now clearly specify that the claimed antibody specifically recognizes the ***phosphorylation site(s)*** of the partial peptide. The partial peptide is a partial peptide of phosphorylated tau protein in a paired helical filament which phosphorylated tau protein comprises the amino acid sequence of SEQ ID No. 1. This amino acid sequence (SEQ ID No. 1) has a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1 and optionally one or more other phosphorylation sites at other positions of SEQ ID No. 1. The partial peptide consists of such phosphorylation site(s) and a plurality of amino acid residues (from SEQ ID No. 1) before and/or after said phosphorylation site(s). Thus, since it is clear that one skilled in the art can discern the peptide immunogen used and the antibody obtained therefrom, this rejection can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1 and 6-17 under 35 USC § 112, first paragraph, as set forth in item 8 of the May 9, 2001 Official Action, this rejection has been overcome by the the wording of the amended claims. Specifically, the claims have been amended to clearly direct to only partial peptides of SEQ ID NO. 1 and to the specific phosphorylation site(s) contained therein which the Examiner has already indicated to satisfy the requirements of 35 USC § 112, first paragraph. Thus, in light of Applicants' amendment to the claims, this rejection can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1 and 6-17 under 35 USC § 102(b) as being anticipated by Vandermeeren et al. (J. of Neruochemistry 61:1828-34, 1993), this rejection is deemed to be untenable in view of the wording of the amended claims and the Rule 1.132 Declaration enclosed herewith, and is thus respectfully traversed.

To constitute anticipation of the claimed invention, a single prior art reference must disclose each and every material element of the claim. Here, in this case, Vandermeeren et al. fail to teach or suggest the antibody specificity and the partial peptides of the amended claims.

The antibody of the present invention, as described in the amended claim 1, is obtained by using, as an immunogen, a partial peptide of phosphorylated tau protein in a paired helical filament. The phosphorylated tau protein comprises the amino acid sequence of SEQ ID No. 1 and has a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1 and optionally one or more other phosphorylation sites at other positions of SEQ ID No. 1. The partial peptide consists of said phosphorylation site(s) and a plurality of amino acid residues (from SEQ ID No. 1) before and/or after said phosphorylation site(s), and the antibody obtained specifically recognizes said phosphorylation site(s) of said partial peptide.

On the other hand, the antibody AT8 of Vandermeeren et al. is obtained by using, as an immunogen, phosphorylated tau protein as a whole. Furthermore, the obtained antibody AT8 recognizes serine at position 202 and threonine at position 205 based on the teachings of M. Goedert et al. and the experiments in the enclosed Declaration. Thus, although Vandermeeren et al. disclose that antibody AT8 recognizes the serines at positions 199-202, based on subsequent experiments, the antibodies ***actually recognizes serine at 202 and threonine at position 205.***

Further, as clearly shown in the Declaration, antibody AT8 does not bind to a **partial peptide** of phosphorylated tau protein in a paired helical filament. Instead, it binds only to paired helical filament tau-protein. Also, the results of cerebrospinal fluid analysis in the Declaration further demonstates how the antibody AT8 differs from that of the present invention. In the

analysis, PS199 assay can distinguish AD patients from control while on the other hand, AT8 assay can not.

Thus, the antibody of the present invention clearly differs from the antibody AT8 of Vandermeeren et al. with respect to the way the antibody is produced and the antibody's specificity to the various phosphorylated sites of the phosphorylated tau protein.

The Examiner states that Vandermeeren et al. teach that the sandwich assay utilizes monoclonal antibody AT8 which recognizes abnormally phosphorylated serines at positions 199-202 in the tau protein.

However, the detection limit for τ was less than 5 pg/ml of CSF using AT8, and Vandermeeren et al. disclose that "when a pool of AD CSF samples was concentrated 12 times, resulting in a hypothetical sensitivity of less than 3 pg/ml PHF-τ, no signal was found. Thus, if PHF-τ, as detected by the AT8 immunoassay, is present in CSF, its concentration must probably be below 3pg/ml." (see page 1831, right column, lines 11-16, of the reference).

Although Vandermeeren et al. disclose a sensitive sandwich ELISA using AT120, AT120 reacts with both phosphorylated and dephosphorylated PHF τ, indicating that its recognizing site is not the phosphorylated site, as described in Fig. 2. The reactivity of the AT120 antibody with PHF-τ was not sensitive to phosphatase treatment either in ELISA (Fig. 2) or on western blots (see page 1829, right column, lines 4-7 from the bottom, of the reference). Furthermore, according to Fig. 4, AT120 reacts with CFS samples from not only the patients of Alzheimer's disease but also the patients suffering from other neurological diseases (OND). Thus, the detection method using AT120 of Vandermeeren et al. is not specific to Alzheimer's disease.

Conversely, the presently claimed antibodies are specific for Alzheimer's disease as shown in the Examples of the specification. The claimed antibodies can be used to detect Alzheimer's disease by examining the reactivity of these antibodies with a body fluid sample from an individual suspected of having Alzheimer's disease. The antibodies of Vandermeeren et al., on the other hand, cannot be used in such a method.

Thus, in light of the above and the enclosed Rule 1.132 Declaration, Applicants believe that this rejection can not be sustained and should be withdrawn.

With regard to the rejections of claims 1 and 6-17 under 35 USC § 102(b) as being anticipated by Kimura et al. (Dementia, 7:177-81, 1996) or Yamaguchi et al. (Acta Neuropathol., 92:232-241, 1996) as set forth in items 15 and 16 of the May 9, 2001 Official Action, these rejections are deemed to be untenable and are thus respectfully traversed.

As noted in Applicants' previous response, the certified copy of the priority document is present in the file and thus, the U.S. filing for the present application is March 13, 1997. Further, with the previous filing of the verified English translation of the certified priority document, Applicants' claim of priority has been perfected and the present application is entitled to a priority date of March 13, 1996.

Applicants have also previously note that the cited references, Kimura et al. and Yamaguchi et al., were published after the priority date (March 13, 1996) of the present application. Applicants have submitted with their response of February 26, 2001, a catalog showing the month of publication for these references. For Kimura et al., the publication date appears to be around August, 1996 and for Yamaguchi et al., the publication date was in July-

August, 1996. Since both of these references have a publication date after March 13, 1996, Applicants submit that Kimura et al. and Yamaguchi et al. are not valid prior art references under 35 USC § 102.

The Examiner in her Official Action dated May 9, 2001 rejected Applicants' argument since the claims appear broader than the embodiments taught by the priority document. The Examiner also noted that Figures 5 and 6 and the data contained therein do not appear to be supported by the priority document.

Applicants believe that the changes to the claims which clarified the claimed subject matter clearly shows that the claims are now supported by the priority document (see claims 1-6 of the English translation of the certified priority document). Further, as discussed during the personal interview, Figures 5 and 6 support aspects of the present invention which was already set forth in the priority document (see the brief descriptions of Figures 5 and 6 on page 12 of the original specification). Thus, the absence of Figures 5 and 6 in the priority document should not preclude the present Application from receiving the benefits of the priority date especially since the disclosure of the priority document clearly support the subject matter of the present claims.

Thus, in view of the above, Applicants respectfully submit that these rejections can no longer be sustained and should be withdrawn.

With regard to the rejection of claims 1 and 6-17 under 35 USC § 102(b) as being anticipated by Biernat et al. (EMBO J., 11(4):1593-97, 1992), this rejection is deemed to be untenable for the same reasons as that argued in the Vandermeeren et al. rejection and is thus, respectfully traversed.

Like Vandermeeren et al., the antibody AT8 of Biernat et al. is obtained by using, as an immunogen peptide, the whole phosphorylated tau protein, and the obtained antibody AT8 recognizes serine at position 202 and threonine at position 205 (see arguments above and in the Rule 1.132 Declaration).

Thus, the antibody of the present invention clearly differs from the antibody AT8 of Biernat et al. in both the way the antibody is produced and the antibody's specificity to the phosphorylated sites of phosphorylated tau protein.

As the Examiner mentioned, Biernat et al. teach that the switch of tau protein to an Alzheimer-like state includes the phosphorylation of two serine-proline motifs upstream of the microtubule binding region. However, it does not describe or suggest the method for detecting Alzheimer's disease by examining reactivity of the antibodies by using, as an immunogen, a partial peptide containing a phosphorylated site of phosphorylated tau protein in the PHF, with a body fluid sample from an individual suspected of Alzheimer's disease.

Thus, in view of the above, Applicants respectfully request that this rejection be withdrawn.

With regard to the rejection of claims 1 and 6-17 under 35 USC § 102(b) as being anticipated by Takahashi et al. (J. of Neurochemistry, 64:1759-68, April 1995), this rejection is deemed to be untenable and is thus, respectfully traversed.

The antibody of Takahashi et al. is directed against phosphorylated serine at position 199 or phosphorylated serine at position 396. Thus, the antibody of the present invention differs from

that of Takahashi et al. with respect to its specificity to the phosphorylation sites of phosphorylated tau protein.

Further, although the Examiner states that Takahashi et al. teach antiserum PS199 antibodies directed to phosphorylated serine 199 and analysis of immunoreactivity in rat brain, it does not teach or suggest the method for detecting Alzheimer's disease by using an antibody that specifically recognizes phosphorylated tau protein of a body fluid sample from an individual suspected of Alzheimer's disease.

Thus, for the same reasons as Vandermeeren et al. and Biernat et al., this rejection should be withdrawn.

Applicants wish to note that although Vandermeeren et al. (1993) and Biernat et al. (1992) indicate that AT8 recognizes abnormally phosphorylated serines at positions 199-202, Goedert et al. (Neuroscience Letters 189 (1995) 167-170) teach that AT8 recognizes the tau protein phosphorylated at both serine 202 and threonine 205. This teaching is significant since the reference, Goedert et al., was published (1995) after Vandermeeren et al. (1993) and Biernat et al. (1992). Also, this teachings is consistent with that shown in the enclosed Rule 1.132 Declaration.

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE**".

In view of the foregoing amendments and remarks, it is respectfully submitted that the Application is now in condition for allowance. Such action is thus respectfully solicited.

If, however, the Examiner has any suggestions for expediting allowance of the application or believes that direct communication with Applicants' attorney will advance the prosecution of this case, the Examiner is invited to contact the undersigned at the telephone number below.

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

The claims have been amended as follows.

1. (Twice Amended) An antibody obtained by using, as an immunogen, a partial peptide ~~comprising two amino acid residues at the phosphorylation sites~~ of phosphorylated tau protein in a paired helical filament ~~and plural~~, said phosphorylated tau protein comprising the amino acid sequence of SEQ ID No. 1 and having a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1, said partial peptide consisting of said serine residue at said phosphorylation site and a plurality of amino acid residues before and/or after ~~the~~said phosphorylation ~~sites of amino acid sequence of SEQ ID NO: 1, wherein the two amino acid residues are threonine at position 231 and serine at position 235, or serine at position 412 and serine at position 413 of amino acid sequence of SEQ ID NO: 1~~ site, and said antibody specifically recognizing said phosphorylation site of said partial peptide.

6. (Twice Amended) A reagent kit for detecting Alzheimer's disease, comprising one or more antibodies obtained by using, as an immunogen, a partial peptide ~~comprising one or more amino acid residue(s) at the phosphorylation sites~~ of phosphorylated tau protein in a paired helical filament ~~and plural~~, said phosphorylated tau protein comprising the amino acid sequence of SEQ ID No. 1 and having a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1, said partial peptide consisting of said serine residue at said phosphorylation site and a plurality of amino acid residues before and/or after ~~the~~said phosphorylation site~~(s) of amino acid sequence of SEQ ID NO: 1, wherein the~~, and said antibodies specifically recognizing said phosphorylation

site(s) are one or more amino acid residue(s) selected from the group consisting of serine at position 198, serine at position 199, threonine at position 231, serine at position 235, serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and serine at position 422 of amino acid sequence of SEQ ID NO: 1 of said partial peptide.

7. (Twice Amended) A method for detecting Alzheimer's disease, comprising examining reactivity ofreacting one or more antibodies from the reagent kit according to claim 6, with a body fluid sample from an individual suspected of having Alzheimer's disease, whereinto detect from the reactivity of said antibodies are obtained by using, as an immunogen, a partial peptide comprising one or more amino acid residue(s) at the phosphorylation sites of phosphorylated tau protein in a paired helical filament and plural amino acid residues before and/or after the phosphorylation site(s) of amino acid sequence of SEQ ID NO:1, wherein the phosphorylation site(s) are one or more amino acid residue(s) selected from the group consisting of serine at position 198, serine at position 199, threonine at position 231, serine at position 235, serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and serine at position 422 of amino acid sequence of SEQ ID NO: 1 whether said individual has Alzheimer.

8. (Amended) The reagent kit according to claim 623, wherein the partial peptide is selected from the group consisting of SEQ ID NO: 2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID

~~NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16~~.

9. (Amended) The method for detecting Alzheimer's disease according to claim ~~7~~24, wherein the partial peptide is ~~selected from the group consisting of~~ SEQ ID NO: 2, ~~SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 16~~.

10. (Amended) An antibody specifically recognizing a partial peptide of a phosphorylated tau protein in a paired helical filament, said phosphorylated tau protein comprising the amino acid sequence of SEQ ID No: 1 and having a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1 and one or more other phosphorylation site(s) at other position(s) of SEQ ID No. 1, said partial peptide ~~comprising one or more phosphorylated sites~~consisting of said ~~phosphorylated tau protein, said phosphorylated site(s) being one or more~~phosphorylation sites and a plurality of amino acid residues ~~of SEQ ID NO: 1 selected from the group consisting of serine at position 198, threonine at position 231, serine at position 235, serine at position 262, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and serine at position 422~~ before and/or after said phosphorylation sites, and said antibody specifically recognizing said phosphorylation sites of said partial peptide.

11. (Amended) The antibody according to claim 10, wherein ~~the partial peptide comprises (a) said phosphorylated sites of (i)~~said one or more other phosphorylation site(s) at other position(s) of SEQ ID No. 1 is selected from the group consisting of serine at position 198, serine at position 202, threonine at position 205, threonine at position 231 ~~and~~, serine at position 235, ~~or (ii)~~serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412 ~~and~~, serine at position 413, and ~~(b) a plurality of amino acid residues before and/or after said phosphorylation sites of the phosphorylated tau protein of SEQ ID NO:1~~serine at position 422.

12. (Not Amended) The antibody according to claim 10, wherein the partial peptide is 1 to 7 amino acid residues in length.

13. (Not Amended) The antibody according to claim 12, wherein the partial peptide is 3 to 5 amino acid residues in length.

14. (Not Amended) The antibody according to claim 10, which is a monoclonal antibody.

15. (Amended) The antibody according to claim 10, wherein the partial peptide is ~~selected from the group consisting of~~ SEQ ID NO: ~~2, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15 and SEQ ID NO: 165~~.

16. (Amended) A reagent kit for detecting Alzheimer's disease comprising one or more antibodies specifically recognizing a partial peptide of a phosphorylated tau protein in a paired helical filament, said phosphorylated tau protein comprising the amino acid sequence of SEQ ID No: 1 and having a phosphorylation site at a serine residue at position 199 of SEQ ID No. 1 and one or more other phosphorylation site(s) at other position(s) of SEQ ID No. 1, said partial peptide ~~comprising one or more phosphorylated sites~~consisting of said ~~phosphorylated tau protein, said phosphorylated site(s) being one or more~~phosphorylation sites and a plurality of amino acid residues ~~of SEQ ID NO: 1 selected from the group consisting of serine at position 198, serine at position 199, threonine at position 231, serine at position 235, serine at position 262, serine at position 396, threonine at position 403, serine at position 404, serine at position 409, serine at position 412, serine at position 413, and serine at position 422.~~ before and/or after said phosphorylation sites, and said antibody specifically recognizing said phosphorylation sites of said partial peptide.

17. (Amended) A method for detecting Alzheimer's disease comprising reacting one or more antibodies from the reagent kit according to claim 1~~0~~6, with a body fluid sample from an individual suspected of having Alzheimer's disease, to ~~determine~~detect from the reactivity of said antibodies whether said individual has Alzheimer.